Competition magnifies the impact of a pesticide in a warming world by reducing heat tolerance and increasing autotomy ☆

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ABSTRACT

There is increasing concern that standard laboratory toxicity tests may be misleading when assessing the impact of toxicants, because they lack ecological realism. Both warming and biotic interactions have been identified to magnify the effects of toxicants. Moreover, while biotic interactions may change the impact of toxicants, toxicants may also change the impact of biotic interactions. However, studies looking at the impact of biotic interactions on the toxicity of pesticides and vice versa under warming are very scarce. Therefore, we tested how warming ($+4$ °C), intraspecific competition (density treatment) and exposure to the pesticide chlorpyrifos, both in isolation and in combination, affected mortality, cannibalism, growth and heat tolerance of low- and high-latitude populations of the damselfly *Ischnura elegans*. Moreover, we addressed whether toxicant exposure, potentially in interaction with competition and warming, increased the frequency of autotomy, a widespread antipredator mechanism. Competition increased the toxicity of chlorpyrifos and made it become lethal. Cannibalism was not affected by chlorpyrifos but increased at high density and under warming. Chlorpyrifos reduced heat tolerance but only when competition was high. This is the first demonstration that a biotic interaction can be a major determinant of 'toxicant-induced climate change sensitivity'. Competition enhanced the impact of chlorpyrifos under warming for high-latitude larvae, leading to an increase in autotomy which reduces fitness in the long term. This points to a novel pathway how transient pesticide pulses may cause delayed effects on populations in a warming world. Our results highlight that the interplay between biotic interactions and toxicants have a strong relevance for ecological risk assessment in a warming polluted world.

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1. Introduction

There is increasing concern that evaluating the impact of pollution under ideal laboratory conditions may be misleading (Liess et al., 2016; Rohr et al., 2016). For example, a striking recent finding was that declines in aquatic biodiversity in Europe have been observed at pesticide concentrations that are regarded as safe by current risk assessment based on laboratory toxicity tests (Beketov et al., 2013). This discrepancy between observations from ecotoxicological testing and natural field situations can be attributed to the presence of environmental stressors that magnify the impact of pesticides in natural systems (Holmstrup et al., 2010; Liess et al., 2016). A recent meta-analysis demonstrated that the presence of both biotic and abiotic stressors can increase the sensitivity to pesticides with a factor of up to 100 (Liess et al., 2016). Warming is such an important abiotic stressor that may interact with pollution and is getting increased attention because of climate change. Exposure to toxicants can impair an organism's ability to cope with warming, and vice versa, warming can change the impact of toxicants (Sokolova and Lannig, 2008; Noyes and Lema, 2015). Examining the impact of toxicants under more realistic ecological conditions is therefore key to develop a better understanding of the impact of toxicants in natural systems in a warming world.

While most multi-stressor studies with toxicants considered abiotic stressors, also stress imposed by biotic interactions may strongly magnify the impact of toxicants (reviewed in Relyea and Hoverman, 2006; Holmstrup et al., 2010; Jackson et al., 2016; Liess et al., 2016). In this context, most attention went to the effect of predation risk (e.g. Relyea and Mills, 2001; Campero et al., 2007), while studies integrating competition are less frequent. Competition, however, is also a major structuring force in aquatic

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communities that may strongly change the impact of toxicants (e.g. Boone and Semlitsch, 2001, Jones et al., 2011; Knillmann et al., 2012). For example, the negative effects of the pesticide esfenvalerate in the water flea *Daphnia magna* increased with a factor up to 100 with increasing intraspecific competition (Knillmann et al., 2012). Biotic interactions are also sensitive to warming. Indeed, organisms typically show increased metabolic and growth rates at higher temperatures, this increases their energetic requirements and often results in an increased foraging effort (Angiletta, 2009). This will increase the encounter rates among species and intensify predator-prey and competitive interactions (Gilman et al., 2010). Therefore, biotic interactions have been identified as crucial to be able to predict the fate of populations under global warming (Gilman et al., 2010; Urban et al., 2016; Stoks et al., 2017). This indicates that it is important to include biotic interactions when assessing the impact of toxicants in a warming world. Although studies like this are rare, there have been a few of such studies. For example, Janssens and Stoks (2017) looked at the effects of predation risk imposed by visual and chemical predator cues, warming and exposure to the pesticide chlorpyrifos on physiological traits. In the study of Knillmann et al. (2013) the influence of warming, interspecific competition, and exposure to the insecticide esfenvalerate on the zooplankton community composition and population recovery was assessed.

Toxicants can also change the impact of biotic interactions just like biotic interactions can change the impact of toxicants. One of the ways by which a toxicant can do this is by changing antipredator mechanisms such as behavioural changes in response to predator cues and changes in escape performance (Teplitsky et al., 2005; Brooks et al., 2009; Van Gossum et al., 2009; Janssens and Stoks, 2012). One widespread antipredator mechanism that has been ignored so far in ecotoxicology is autotomy, where an animal sacrifices a body part to escape predation (Fleming et al., 2007). While autotomy has immediate survival benefits, it is costly in the long term as it will increase the vulnerability to future encounters with predators and energy is needed to regrow the autotomized body part (Fleming et al., 2007). Autotomy increases with the number of encounters with predators, and animals may become more active when exposed to toxicants (e.g. Janssens and Stoks, 2012) and warming (e.g. Janssens et al., 2014) since they need to forage more in order to meet the higher energy demand for detoxification and damage repair under pesticide exposure (Congdon et al., 2001), and to sustain their higher metabolism under warming (Angiletta, 2009). It may therefore be expected that both stressors increase the frequency of autotomy (for warming: Start et al., 2017). As such, autotomy may be an overlooked mechanism how transient exposure to toxicants generates delayed costs.

The aim of this study was to test the effect of intraspecific competition on the sensitivity of an aquatic insect to toxicants under global warming. We therefore documented in a common garden warming experiment how warming, competition and pesticide exposure in isolation and in combination affected mortality, growth and heat tolerance. Moreover, we addressed whether toxicant exposure, potentially in interaction with competition and warming, increased the frequency of autotomy. We studied this in the damselfly *Ischnura elegans*. Damselflies are important intermediate predators in aquatic systems (Stoks and Córdoba-Aguilar, 2012), and are vulnerable to both global warming (Hassall and Thompson, 2008) and toxicant exposure (Liess and Von der Ohe, 2005), since the obligate aquatic stage cannot escape exposure to these stressors. Competition is an important biotic interaction in larval damselfly populations (e.g. Anholt, 1990; McPeek and Peckarsky, 1998), and damselfly larvae rely on autotomy of their caudal lamellae to escape predation (Stoks, 1998a). We exposed *I. elegans* larvae from replicated high- and low-latitude populations

of this species in Europe in a common garden warming experiment. Common garden experiments are a standard method to demonstrate local adaptation (Kawecki and Ebert, 2004). In this type of experiments organisms from both latitudes are reared under common conditions with the manipulation of one environmental variable for which local adaptation is tested. In our case we reared animals from both latitudes under common conditions either at the mean summer water temperature of the high latitude (20 °C) or the low latitude (24 °C). This approach allows testing how the latitude-associated thermal adaptation in the species (De Block et al., 2013; Shama et al., 2011; Op de Beeck et al., 2017a), and the latitudinal differences in life history (the low-latitude larvae grow and develop faster, Shama et al., 2011; Stoks et al., 2012) may generate changes in the sensitivity to toxicants. For this study, we used chlorpyrifos as the toxicant of interest. Chlorpyrifos is an organophosphate and one of the most frequently used insecticides worldwide (Eaton et al., 2008) and a priority pollutant in the European Water Framework Directive (2000/60/EC). We choose to apply the pesticide under an environmental realistic multiple pulse scenario without renewal of the medium, thereby mimicking as series of releases/emissions of a pesticide in a pond. For the study species, the sensitivity to CPF increases under warming as evidenced when the CPF concentration is kept constant (Dinh Van et al., 2014), yet, the net impact of CPF under warming may decrease under a multiple pulse scenario because of higher pesticide degradation and less pesticide accumulation (Op de Beeck et al., 2017a).

2. Material & methods

2.1. Collecting and housing

Mated females (12–15 per population) of the damselfly *I. elegans* were collected from replicated populations in the low-latitude and high-latitude regions of the species' distribution in Europe (Gosden et al., 2011). Per latitude three populations were chosen randomly and sampled end June – early July 2014. For the low-latitude region (southern France) we sampled at Saint-Martin-de-Crau (43°38′16.61″N, 4°50′49.05″E), Camaret-sur-Aigues (44°9′1.50″N, 4°51′20.36″E), and Valcros (43°10′9.06″N, 6°16′11.36″E). For the high-latitude region we sampled the Danish population Ahl Hage (56°10′59.66″N, 10°39′1.69″E) and the Swedish populations Nöbelövs mosse (55°44′6.00″N, 13°9′10.00″E) and Eriksö (58°56′4.90″N, 17°39′21.50″E). All sampling sites were shallow lakes with a rich aquatic vegetation. Details on the study sites can be found in Op de Beeck et al. (2017b).

To obtain eggs, mated females were placed individually in plastic cups with wet filter paper as substrate for oviposition. The deposited eggs were transferred to the lab in Belgium, where they were incubated. Larvae were reared individually in 200 ml plastic cups filled with 100 ml dechlorinated water at one of two water temperatures (20 °C or 24 °C) and a photoperiod of L:D 14:10 h until they reached the final instar. For more details on the housing of damselfly larvae see Op de Beeck et al. (2017b). One day after their moult into the final instar, larvae were introduced in the pesticide exposure experiment at their respective rearing temperature. In this way all larvae had been acclimated to their experimental temperature (starting from the egg stage) for more than 14 weeks before we tested effects of the pesticide and competition. This also ensured that all larvae were standardized for the same "physiological age" when the exposure started. The chosen temperatures match the average summer water temperature of the water bodies inhabited by the study species at low (24 °C) and high (20 °C) latitude in Europe (De Block et al., 2013). Moreover, the 4 °C temperature difference matches the predicted warming at high latitudes by the year 2100 under IPCC (IPCC, 2013) scenario RCP 8.5.

During the rearing period larvae received *Artemia* nauplii (206 ± 10 nauplii per food portion, mean ± SE, n = 41 food portions) six days a week.

2.2. Experimental design

To study the effects of pesticide exposure, warming and density (to manipulate intraspecific competition) on life history traits and heat tolerance, and how these differ between latitudes, we ran a full factorial experiment in microcosms with two latitudes × two pesticide treatments (chlorpyrifos absent and present) × two temperatures (20 and 24 °C) × two density treatments (low and high). We started with 19—22 microcosms per combination of latitude × pesticide treatment × temperature for the low density treatment and 9 to 11 microcosms per treatment combination for the high density treatment (total of 252 microcosms, 945 larvae). More microcosms were started for the low density treatment in order to obtain enough larvae to measure the response variables. Exact initial numbers of larvae per treatment are given in Fig. 1A. Surviving larvae were used to estimate growth rate and heat tolerance. Afterwards, larvae were frozen at −80 °C for physiological analyses. Exact final sample sizes for each variable are presented in the figures.

The day after the larvae molted into the final instar we started the 7-day pesticide exposure period. Larvae were transferred to circular, glass microcosms (diameter 25 cm) holding 1 L of aged dechlorinated tap water. Larvae were placed in groups of two (low density) or seven/eight (high density) per microcosm depending on the competition treatment. The initial number at high density slightly differed between replicates depending on the availability of newly moulted larvae to start up a replicate. These densities are within the range of densities observed for coenagrionid damselfly larvae in natural populations (McPeek, 1990; Corbet, 1999). To further reflect the natural situation, all larvae in a microcosm originated from the same population of origin. Larvae were tracked individually in each microcosm by marking the legs with a specific, individual pattern with a permanent marker (Staedtler Lumocolor 313). The handling to apply markings and the markings themselves had no effect on mortality when compared with animals that did not experience the marking procedure (Lin Op de Beeck, personal observation).

Each microcosm, independent of the density (as in e.g. McPeek and Crowley, 1987; Boone and Semlitsch, 2002, Jones et al., 2011), received the same amount of *Artemia* (824 ± 40 nauplii per food portion, mean ± SE, n = 41 food portions) seven days a week. This means there were ca. 4x more *Artemia* available per larva per day in the low compared to the high density treatment. By using a fixed amount of food items for the two density treatments we can, besides interference competition, simulate exploitation competition. In order to keep the intensity of competition for food constant during the exposure period we adjusted the amount of food when larvae died, so that the number of *Artemia* per larva per day remained the same throughout the exposure period. In the high density treatment there were always uneaten food items when the new food portion was given indicating that even for this treatment food was not limiting.
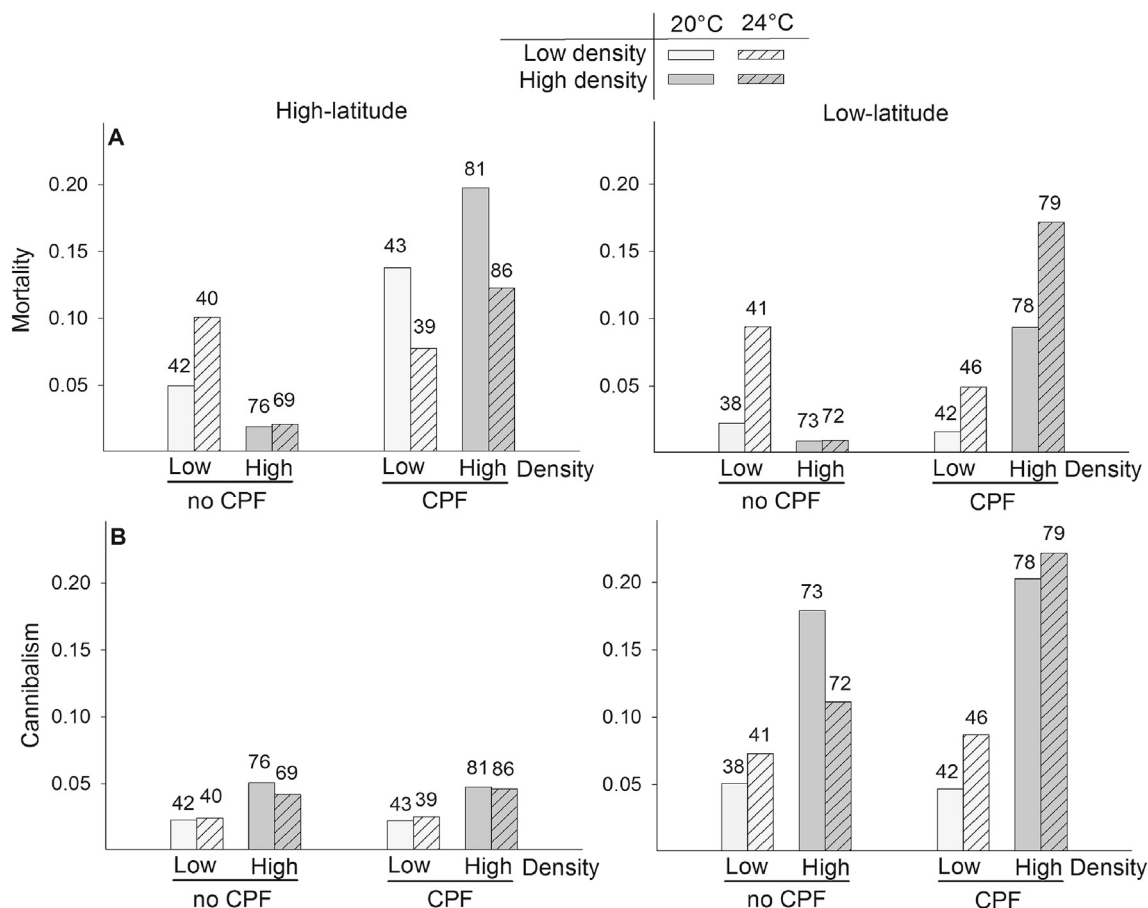
Fig. 1. (A) Mortality (proportion intact dead larvae per microcosm) and (B) cannibalism (proportion (partly) eaten dead larvae per microcosm) of *Ischnura elegans* larvae as a function of chlorpyrifos (CPF) exposure, rearing temperature, latitude of origin and density. Numbers above bars give number of larvae tested.

2.3. Pesticide treatment

Larvae were subjected to a 7-day exposure period to the pesticide chlorpyrifos (CPF). The exposure consisted of three sequential pesticide pulses, every 72 h, applied without renewal of the medium. The measured concentration of each pulse (and initial concentration after the first pulse) was 1.1 µg L^{-1}; this was based on a pooled sample of the medium of 10 microcosms. Pesticide quantification in water samples was carried out by the research laboratory Lovap NV (Geel, Belgium) by use of gas chromatography in combination with mass spectrometry. The chosen pulse concentration was based on the study by Dinh Van et al. (2014) where a CPF concentration of 1.5 µg L^{-1} caused a growth reduction in *I. elegans* larvae in a static renewal 6-day exposure experiment. Note that in the current experiment because of no renewal of the medium accumulation of CPF may occur. Moreover, higher pesticide degradation rates and possibly also lower pesticide accumulation at higher temperatures may lead to lower exposure concentrations under warming (see e.g. Op de Beeck et al., 2017a; Op de Beeck et al., 2017b). The chosen pulse concentration is rather high but realistic in water bodies near agricultural lands where the study species can be very abundant (Dijkstra, 2006) and where peak concentrations can exceed 100 µg L^{-1} due to runoff (Moore et al., 2002; Mazanti et al., 2003; Bernabò et al., 2011). A multiple pulse scenario is realistic in ponds in agricultural areas since most common pesticides are applied multiple times during the growth season of crops, and when combined with rainfall this will lead to multiple run-off events (Dabrowski et al., 2002).

To assess the breakdown of CPF at both temperatures, a pooled sample of 20 microcosms was taken at each temperature treatment after 72 h (just before the second pesticide pulse). The concentration after 72 h at 20 °C was 0.08 µg L^{-1} while at 24 °C the concentration dropped below the detection limit of 0.05 µg L^{-1}. To assess any accumulation of CPF at both temperatures, water samples were taken immediately after the third (= final) pesticide pulse. This concentration was 1.3 µg L^{-1} at 20 °C, and 1.0 µg L^{-1} at 24 °C, suggesting a slower degradation, hence higher accumulation of CPF at 20 °C (as documented in Op de Beeck et al., 2017a). This reflects the known higher degradation rate of pesticides at higher temperature (Lartiges and Garrigues, 1995).

2.4. Response variables

Mortality was checked every day during the 7-day pesticide exposure period. For every animal that died we documented whether it was intact and died from pesticide exposure or if the body was partly eaten indicating that the animal died from cannibalism. Damselfly larvae are cannibalistic (De Block and Stoks, 2004). Each larva was weighed to the nearest 0.01 mg using an electronic balance (AB135-S, Mettler Toledo®, Ohio, USA) before (initial wet mass) and after (final wet mass) the exposure period. This was used to calculate growth rate during the exposure period as [ln (final mass) - ln (initial mass)]/7 days (Stoks et al., 2012). Wet mass estimates were obtained by gently blotting larvae dry with tissue paper before weighing; these have been shown to strongly correlate with dry mass (Stoks et al., 2005). For every surviving larva the number of remaining caudal lamellae at the end of the experiment was recorded. This response variable informs about the strength of aggressive interactions, hence interference competition, between damselfly larvae (Stoks, 1998b; Witt et al., 2013). Damselfly larvae have three caudal lamellae and can autotomize these when grasped by conspecific and heterospecific predators (Stoks and De Block, 2000). The initial number of lamellae per larva at the start of the exposure experiment was always three.

To estimate the heat tolerance we quantified the critical thermal maximum (CTmax). This measure is proven to be a good proxy for an organism's vulnerability to climate warming (Huey et al., 2012). We used the dynamic method (Verberk and Bilton, 2013) where animals were heated at a constant rate of 0.3 °C/min until they reached CTmax. This is the temperature where animals no longer show any body movements or muscular spasms (Lutterschmidt and Hutchison, 1997). The detailed protocol for CTmax quantification is described in Op de Beeck et al. (2017b).

2.5. Statistical analyses

We tested for effects of the pesticide, temperature, latitude, density and their interactions on the different response variables using a set of linear mixed models. We added population nested within latitude and the microcosm identity to each model as random effects; this way we took into account that larvae from the same microcosm are not independent. Given that mortality (both from cannibalism and the pesticide) is a binary response variable, we analysed it using generalized linear mixed models with a binomial error distribution and logit-link function. For CTmax the larval mass was included as a covariate since the mass of aquatic invertebrates affects their thermal tolerance (Kelly et al., 2014). We analysed growth rate and all physiological variables using linear mixed models with a normal error structure and the identity link. Whenever there was a significant interaction we tested which means differed using Tukey HSD posthoc tests. All analyses were performed with R v3.1.2 for Windows (R Core Team, 2014) using the 'lme4' package for constructing linear mixed models (Bates et al., 2012). To compute Wald chi-square statistics and p-values for fixed effects we use the package 'car' (Fox and Weisberg, 2010).

3. Results

3.1. Mortality and cannibalism

Exposure to CPF increased mortality ($\chi^2_1 = 10.20$, $P = 0.0014$), but only at the high density treatment (Pesticide × Density, $\chi^2_1 = 12.10$, $P < 0.001$; Tukey test for pesticide effect at high density: $P < 0.001$, at low density: $P = 0.92$; Fig. 1A). Temperature and latitude, nor their interactions were significant (all $P > 0.13$).

Cannibalism was higher in low-latitude larvae than high-latitude larvae ($\chi^2_1 = 18.51$, $P < 0.001$) and higher at high density than at low density ($\chi^2_1 = 12.10$, $P < 0.001$, Fig. 1B). CPF exposure and temperature, nor their interactions were significant (all $P > 0.23$).

3.2. Growth rate

Growth rate was higher at low than at high density ($\chi^2_1 = 112.85$, $P < 0.001$). Low-latitude larvae had a higher growth rate than high-latitude larvae ($\chi^2_1 = 33.62$, $P < 0.001$), but only at low density (Latitude × Density, $\chi^2_1 = 11.25$, $P < 0.001$; Tukey test for latitude effect at low density: $P < 0.001$, at high density: $P = 0.082$; Fig. 2). The higher growth rate of low-latitude compared to high-latitude larvae was more pronounced at 24 °C ($P < 0.001$) than at 20 °C ($P = 0.037$) (Latitude × Temperature, $\chi^2_1 = 7.34$, $P = 0.0068$; Fig. 2). In accordance with the significant Latitude × Temperature interaction, high-latitude larvae tended to have a lower growth rate under 4 °C warming ($P = 0.074$), while low-latitude larvae tended to have a higher growth rate under warming ($P = 0.059$). The effect of CPF on growth rate differed significantly between latitudes (Latitude × Pesticide interaction, $\chi^2_1 = 6.61$, $P = 0.010$; Fig. 2). While high-latitude larvae if anything tended to reduce growth rate under pesticide exposure ($P = 0.10$), low-latitude larvae tended to increase growth rate ($P = 0.077$).
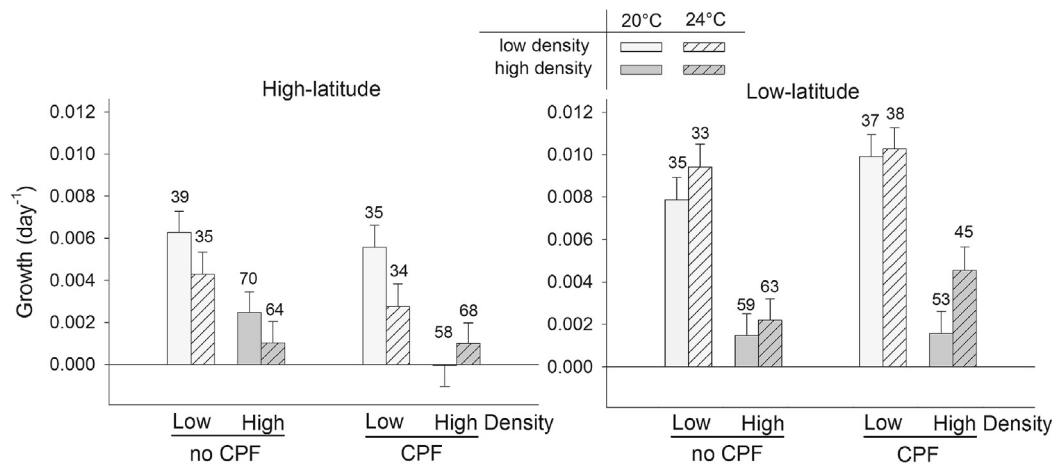
Fig. 2. Growth rate of *Ischnura elegans* larvae as a function of chlorpyrifos (CPF) exposure, rearing temperature, latitude of origin and density. Least square means are given with 1 SE. Numbers above bars give number of larvae tested.

3.3. Number of caudal lamellae

At the end of the 7-day exposure period low-latitude larvae had less lamellae than high-latitude larvae ($\chi^2_1 = 33.22$, $P < 0.001$; Fig. 3). Larvae had less lamellae at high density than at low density ($\chi^2_1 = 19.55$, $P < 0.001$; Fig. 3). The number of lamellae was lower at 24 °C than at 20 °C but only for larvae exposed to CPF at high density ($P < 0.001$, all other groups $P > 0.15$; Temperature $\times$ Pesticide $\times$ Density, $\chi^2_1 = 4.49$, $P = 0.034$), and for low-latitude larvae at high density ($P = 0.0024$, all other groups $P > 0.12$; Temperature $\times$ Latitude $\times$ Density, $\chi^2_1 = 5.41$, $P = 0.020$; Fig. 3). Exposure to CPF resulted in a reduced number of lamellae, but only for high-latitude larvae at 24 °C and high density ($P = 0.031$, all other groups $P > 0.12$; Pesticide $\times$ Temperature $\times$ Latitude $\times$ Density, $\chi^2_1 = 4.92$, $P = 0.027$; Fig. 3).

3.4. Heat tolerance (CTmax)

Exposure to warming resulted in an increased CTmax ($\chi^2_1 = 76.00$, $P < 0.001$, Fig. 4). Exposure to CPF led to a decrease in CTmax, however, only when larvae were exposed to the pesticide at 20 °C ($P < 0.001$) and not at 24 °C ($P = 0.12$, Pesticide $\times$ Temperature, $\chi^2_1 = 5.03$, $P = 0.025$; Fig. 4). The negative effect of CPF was also dependent on the density treatment ($\chi^2_1 = 5.88$, $P = 0.015$, Fig. 4), with CPF exposure causing a reduction in CTmax at high density ($P < 0.001$) but not at low density ($P = 0.11$). The heat tolerance was not affected by latitude or its interactions (all $P > 0.093$).

4. Discussion

Exposure to CPF negatively affected survival, heat tolerance, and resulted in higher levels of autotomy (as evidenced by less remaining caudal lamellae). These effects, however, strongly depended on density and temperature and to a lesser extent on latitude. Our results thereby illustrate the need for realistic conditions in ecological risk assessment of pesticides.

4.1. Density effects

At high density larvae were suffering increased intraspecific competition as evidenced by the lower growth rate (see also Therry
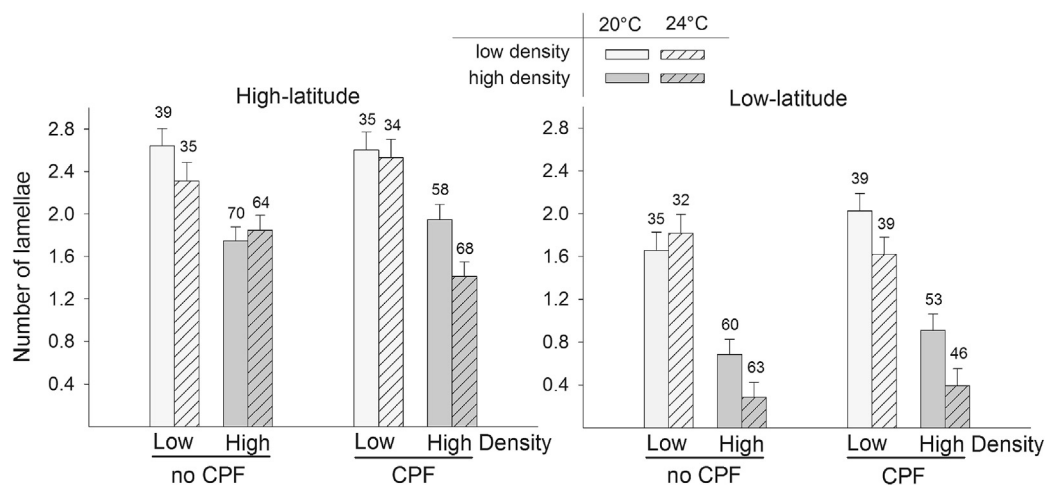
Fig. 3. Number of caudal lamellae of *Ischnura elegans* larvae at the end of the exposure period as a function of chlorpyrifos (CPF) exposure, rearing temperature, latitude of origin and density. The initial number of lamellae per larva at the start of the exposure experiment is three. Least square means are given with 1 SE. Numbers above bars give number of larvae tested.
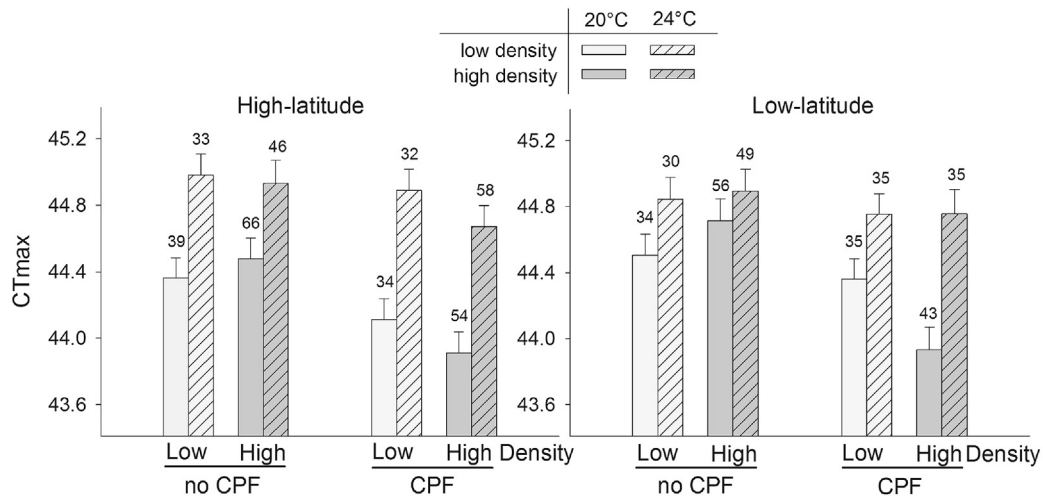
Fig. 4. Heat tolerance (CTmax) of *Ischnura elegans* larvae as a function of chlorpyrifos (CPF) exposure, rearing temperature, latitude of origin and density. Least square means are given with 1 SE. Numbers above bars give number of larvae tested.

et al., 2016). As food was apparently not limiting (see methods), this took the form of interference competition for space rather than exploitation competition for food. This matches previous observations in damselfly larvae (Anholt, 1990; McPeek and Crowley, 1987). In line with this we also observed at the high density the expected increased cannibalism (see e.g. also McPeek and Crowley, 1987; Van Buskirk, 1989) and more missing caudal lamellae (see e.g. also Robinson et al., 1991). Both density patterns reflect the more frequent aggressive encounters among larvae at high density (McPeek and Crowley, 1987; Nilsson-Örtman et al., 2014).

4.2. Latitudinal differences in life history and heat tolerance

Linked to the higher voltinism in low-latitude populations (Corbet et al., 2006), low-latitude larvae had a higher growth rate in order to complete multiple generations per growing season (Corbet et al., 2006; Shama et al., 2011). In order to sustain this fast lifestyle under the experienced time stress, low-latitude larvae forage more and have a higher activity and food intake (Stoks et al., 2012; Dinh Van et al., 2013; Janssens et al., 2014; Debecker et al., 2017). These patterns have been described before in several other species that show more generations per growing season at low latitudes (e.g. other damselfly species: Śniegula et al., 2012; butterflies: Nygren et al., 2008; Seiter and Kingsolver, 2013; mosquitoes: Ragland and Kingsolver, 2007). This more active life style at low latitudes is expected to lead to more aggressive encounters with conspecifics and may explain both the higher cannibalism and higher lamellae loss in low-latitude larvae. Similarly, a higher time stress caused a faster life history and more cannibalism in other damselfly species (Johansson and Rowe, 1999; De Block and Stoks, 2004; Sniegula et al., 2017). Individuals showing cannibalism have a double advantage: they do not only obtain high-quality nutrition, yet at the same time eliminate competitors (Wissinger et al., 1996; Wagner et al., 1999). In this way cannibals can obtain a growth advantage over non-cannibals (Persson et al., 2000; De Block and Stoks, 2004). The higher growth of low-latitude larvae disappeared at high density, likely because the competitive interactions among low-latitude larvae became so stressful at high density that a high growth rate could not be sustained anymore.

We detected a signal for latitude-associated thermal adaptation for growth rate, with larvae from a certain latitude growing faster at their locally encountered temperature. Indeed, low-latitude larvae tended to grow better at 24 °C (the mean summer water temperature at that latitude, De Block et al., 2013) than at 20 °C, while the opposite was true for the high-latitude larvae that tended to grow better at 20 °C than at 24 °C. Patterns of local thermal adaptation along a latitudinal gradient have been observed before in several taxa (e.g. Liefting et al., 2009; De Block et al., 2013; Op de Beeck et al., 2017a). This beneficial effect of warming on growth only being present in low-latitude larvae may explain why only for these larvae warming resulted in less lamellae at high density (as they likely forage more under warming to fuel the growth increase, Stoks et al., 2012). The latitude-associated thermal adaptation did, however, not result in a higher heat tolerance in low-latitude larvae. This matches other studies that found little geographic variation in upper thermal limits (Addo-Bediako et al., 2000; Liefting et al., 2009; Hoffmann et al., 2013; but see Rezende et al., 2014). The higher rearing temperature did result in a higher heat tolerance, confirming the general acclimation pattern described in the literature (e.g. Rajaguru, 2002; Dallas and Rivers-Moore, 2012).

4.3. Pesticide effects on life history and heat tolerance

Exposure to CPF increased mortality but only at high density. This supports the view that increased competition at higher densities can lead to a higher toxicity of pesticides (e.g. Boone and Semlitsch, 2002, Jones et al., 2011; Knillmann et al., 2012; Dinh et al., 2016). Both biotic and abiotic factors can alter the sensitivity of organisms to toxicants (Heugens et al., 2001; Holmstrup et al., 2010; Liess et al., 2016), this so called context sensitivity (Liess and Beketov, 2011) can substantially differ from the toxicological sensitivity estimated under favorable lab conditions (cf. Tier 1 approach). These environmental factors typically reduce an organism's energy budget so that less energy is available for detoxification and damage repair, leading to more severe effects of the toxicant than when exposed to it in isolation (Liess et al., 2016).

We found little signal of latitude-specific effects of CPF, except for a differential effect of CPF on growth rate. While CPF tended to reduce growth rate in high-latitude larvae, it tended to increase growth rate in low-latitude larvae. CPF-induced growth reductions are documented in several taxa (e.g. Widder and Bidwell, 2006; Huynh and Nugegoda, 2012), including damselfly larvae (e.g. Arambourou and Stoks, 2015). The latitudinal pattern suggests lower sensitivity of low-latitude larvae to CPF, thereby supporting

two other studies on *I. elegans* (Op de Beeck et al., 2017b; Op de Beeck et al., 2017a). In line with this, low-latitude larvae have a higher baseline activity of glutathione-S-transferase (an important enzyme in detoxification) and of acetylcholine esterase (the target enzyme of CPF) (Op de Beeck et al., 2017a). In addition, since cannibalism was higher in the low-latitude larvae compared to the high-latitude larvae, also density-mediated effects and competitive release may have resulted in an increased growth rate in this treatment group under CPF exposure (e.g. Liess, 2002).

For the high-latitude larvae, exposure to CPF resulted in less lamellae, hence more autotomy, and reflects more antagonistic encounters. However, more autotomy was observed in the high-latitude larvae only at high temperature and high density. This effect of CPF being only present in high-latitude larvae may reflect their higher sensitivity to CPF. The more sensitive high-latitude larvae may have needed to invest more in energetically costly detoxification and damage repair (Congdon et al., 2001), resulting in more activity and encounters with conspecifics, hence a higher need to use autotomy, especially under warming and high density. Furthermore, warming resulted in less lamellae but only at high density under CPF exposure. Also this may reflect the higher energy need which was likely the highest under these conditions. We acknowledge, however, that in the absence of direct measurements of energy budgets this remains conjecture and awaits formal testing. Whatever the specific mechanism, a higher proportion of autotomy is an important, overlooked ecological mechanism how pollutants may cause delayed fitness costs. Indeed, lamellae autotomy despite being beneficial in the short-term for survival results in a slower escape swimming and therefore higher vulnerability to predation (Stoks, 1998a; Gyssels and Stoks, 2005) and a reduced foraging success (Stoks, 1999) leading to smaller adults (Stoks, 2001) thereby reducing fitness (Stoks & Cordoba-Aguilar 2012). Moreover, autotomy reduces immune function and antioxidant defense (Slos et al., 2009).

A reduction in heat tolerance (CTmax) after exposure to toxicants is widespread (e.g. in vertebrates: Heath et al., 1994; Patra et al., 2007; in invertebrates: Bagwe et al., 2015; Op de Beeck et al., 2017b) and is central to the concept of 'toxicant-induced climate change sensitivity' (Noyes and Lema, 2015). Exposure to a toxicant increases the energy demand and therefore lowers the aerobic scope (the excess capacity to deliver oxygen, Verberk et al., 2016), leading to reduced heat tolerance (Sokolova and Lannig, 2008). In current study, the CPF-induced reduction in CTmax was only present when larvae were exposed at 20 °C, likely reflecting the lower degradation and higher accumulation of CPF at 20 °C compared to 24 °C (see methods and Op de Beeck et al., 2017b). As a consequence, under the here applied multiple pulse scenario of CPF, the predicted temperature increase of 4 °C under IPCC (2013) scenario RCP8.5 will reduce the impact of this pesticide on heat tolerance (see also Op de Beeck et al., 2017b). Notably, CTmax was also reduced after CPF exposure at high density but not at low density. This may be explained by the higher energy demand at high density resulting from the more intense competition. This points to a so far ignored pathway how biotic interactions (here competition) can shape the effects of pesticides.

5. Conclusions

Our results highlight the interplay between biotic interactions and the impact of pesticides with strong relevance for risk assessment in a warming polluted world. On the one hand, competition with conspecifics magnified the impact of the pesticide CPF with as striking result that CPF only caused mortality and a reduced heat tolerance in the presence of competition. Notably, the latter is the first demonstration that a biotic interaction can be a major determinant of 'toxicant-induced climate change sensitivity' (Noyes and Lema, 2015). This adds an important dimension to the recent insight that we need considering biotic interactions to assess the fate of populations under global warming (Urban et al., 2016; Stoks et al., 2017) as we here show that competition may strongly change heat tolerance in a polluted world. On the other hand, we identified a novel pathway how exposure to CPF may shape biotic interactions, namely by increasing the occurrence of autotomy, a widespread antipredator mechanism, which in the long term may make animals more vulnerable to predation and reduce fitness (Fleming et al., 2007). This is an unexplored mechanism how transient pesticide pulses may cause delayed effects on populations.

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